

CTX-M-5, a Novel Cefotaxime-Hydrolyzing β -Lactamase from an Outbreak of *Salmonella typhimurium* in Latvia

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At a children's hospital in Riga, Latvia, isolates identified as *Salmonella typhimurium* were found to be resistant to expanded-spectrum cephalosporins. Two of the resistant strains were analyzed for the mechanism of cephalosporin resistance. Isoelectric focusing revealed a common β -lactamase with a pI of 8.8. In addition, one of the strains produced a pI 7.6 β -lactamase. A transconjugant producing only the pI 7.6 enzyme was susceptible to expanded-spectrum cephalosporins; therefore, this enzyme was most likely SHV-1. Transformants producing only the pI 8.8 β -lactamase were resistant to cefotaxime and aztreonam but were susceptible or intermediate to ceftazidime. A substrate profile determined spectrophotometrically with purified enzyme revealed potent activity against cefotaxime, with a relative k_{cat} value of 95 (benzylpenicillin equal to 100). The enzyme showed lower relative k_{cat} values for ceftazidime (3.3) and aztreonam (9.3). In addition, the enzyme was inhibited by clavulanate, sulbactam and tazobactam, with 50% inhibitory concentrations of 19, 100, and 3.4 nM, respectively. These results indicated the presence of an unusual extended-spectrum β -lactamase. The gene expressing the pI 8.8 β -lactamase was cloned. Nucleotide sequencing revealed a β -lactamase gene that differs from the gene encoding CTX-M-2, which also originated from *S. typhimurium*, by 11 nucleotides, 4 of which result in amino acid substitutions: Ala27Thr, Val230Gly, Glu254Ala, and Ile278Val. These results indicated the presence of a novel extended-spectrum β -lactamase, designated CTX-M-5, that specifically confers resistance to cefotaxime.

Resistance to expanded-spectrum β -lactam antibiotics in the *Enterobacteriaceae* is often due to the presence of extended-spectrum β -lactamases (ESBLs), which are most often derivatives of TEM or SHV enzymes (11, 17). However, there is a small but growing family of plasmid-mediated ESBLs, including CTX-M-1 (MEN-1) (2, 4, 5), CTX-M-2 (3, 5), CTX-M-3 (13), CTX-M-4 (12), Toho-1 (15), and Toho-2 (18), that preferentially hydrolyze cefotaxime. These enzymes are not closely related to TEM or SHV β -lactamases (5) but are all members of Ambler's class A. ESBLs have been found previously in strains of *Salmonella* spp. including *Salmonella typhimurium* strains from Turkey (29), Argentina (3, 26), Tunisia (6), Spain (20), and Russia (12). Two of these reports described the CTX-M-2 and the CTX-M-4 β -lactamases (3, 12).

A large outbreak of gastroenteritis caused by *Salmonella* sp. has occurred among children in Latvia. Over 4,000 cases were reported from 1991 through the first quarter of 1998. Approximately 70% of these patients were under 1 year old, and the illnesses were moderate to severe, with bloody stools and high fever. Some of the cases were complicated by extraintestinal infections. The epidemiology of this outbreak is currently under investigation. In addition to the hospitalized patients, some of the cases occurred among babies residing in orphanages. The clinical isolates of *S. typhimurium* associated with the majority of these infections were particularly noteworthy because of their increased resistance to expanded-spectrum

β -lactam antibiotics, especially cefotaxime. In this study, we report a new member of the CTX-M cefotaxime-hydrolyzing β -lactamase family, designated CTX-M-5.

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MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in this study are listed in Table 1. *S. typhimurium* strains were cefotaxime-resistant clinical isolates obtained from individual patients at Children's Hospital, Riga, Latvia. *Escherichia coli* DH5 α and *E. coli* DH5 α /pCLL2300 were used in transformation, transconjugation, and cloning experiments.

Identification and susceptibility tests. The isolates were identified initially as *S. typhimurium* in the microbiology laboratory of the Children's Hospital Medical Academy of Latvia. This identification was confirmed in the reference laboratory by the API-20E identification system (bioMérieux-Vitek, Hazelwood, Mo.). MICs of various β -lactam antibiotics were determined with broth microdilution tests by standard methods (21). The following antibiotics were used: piperacillin and tazobactam (Lederle Laboratories, Pearl River, N.Y.); ticarcillin and potassium clavulanate (Beecham Laboratories, Bristol, Tenn.); sulbactam (Pfizer Inc., New York, N.Y.); ceftazidime (Glaxo Group Research Ltd., Greenford, England); cefotaxime (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.); imipenem (Merck, Rahway, N.J.); aztreonam, cefepime, and benzylpenicillin (Bristol-Myers Squibb, Princeton, N.J.); cephaloridine (Eli Lilly, Indianapolis, Ind.); cefotetan (Zeneca Pharmaceuticals, Wilmington, Del.); and ceftioxin (Sigma Chemical Company, St. Louis, Mo.). Conditions used for testing β -lactam- β -lactamase inhibitor combinations were those recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (21): ampicillin-sulbactam, 2:1 ratio of drug to inhibitor; ticarcillin-clavulanate, constant concentration of 2 μ g/ml of inhibitor; piperacillin-tazobactam, constant concentration of 4 μ g/ml of inhibitor. Susceptibility to non- β -lactam antibiotics was determined by a disk diffusion assay (22).

IEF and β -lactamase assays. Crude preparations of β -lactamases from clinical isolates were obtained by repeated freezing-thawing in 0.02 M sodium acetate, pH 5.2. Isoelectric focusing (IEF) was performed by the method of Matthew et al. (19) by using an LKB Multiphor apparatus with prepared PAGplates, pH 3.5 to 9.5 (Pharmacia LKB, Piscataway, N.J.). The isoelectric point (pI) of each

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TABLE 1. Bacterial strains used in this study

Strain	Organism	Plasmid	Characteristics	pI of β -lactamase(s)	Source or reference
28	<i>S. typhimurium</i>	pCLL3425	Clinical isolate	8.8	This study
34	<i>S. typhimurium</i>	pCLL3422 + pCLL3425	Clinical isolate	7.6, 8.8	This study
DH5 α	<i>E. coli</i>		<i>E. coli</i> K-12 cloning strain	None	14
			F ϕ 80dlacZ Δ M15 Δ (<i>lacZYA-argF</i>) U169 <i>recAI</i> <i>endAI</i> <i>hsdR17</i> (r_K^- m_K^+) <i>supE44</i> <i>thiI</i> <i>gyrI</i> <i>relAI</i>		
DH5 α	<i>E. coli</i>	pCLL2300	Km ^r cloning vector	None	24
DH5 α	<i>E. coli</i>	pCLL3422 + pCLL2300	Transconjugant of <i>S. typhimurium</i> 34	7.6	This study
DH5 α	<i>E. coli</i>	pCLL3425	Transformant of <i>S. typhimurium</i> 34	8.8	This study
DH5 α	<i>E. coli</i>	pCLL3417	7-kb <i>Bam</i> HI fragment containing <i>bla</i> _{CTX-M-5} in pCLL2300	8.8	This study

enzyme was confirmed by activity staining with nitrocefin (Becton Dickinson Microbiology Systems, Cockeysville, Md.) following IEF. The pI of CTX-M-5 was calculated by using β -lactamase standards with known isoelectric points.

E. coli DH5 α /pCLL3417, which produces the pI 8.8 CTX-M-5 β -lactamase from cefotaxime-resistant *S. typhimurium*, was used for further enzymatic analysis. CTX-M-5 β -lactamase crude extracts were obtained from 2 liters of log-phase cells in Trypticase soy broth after five cycles of freezing-thawing in 0.02 M sodium acetate followed by 30 min of ultracentrifugation at 100,000 $\times g$ using a Beckman L8-M ultracentrifuge. This enzyme extraction was initially eluted from a Sephadex G-75 column equilibrated with 50 mM phosphate buffer, pH 7.0. Fractions were monitored at 280 nm for protein and tested for β -lactamase hydrolytic activity with 50 μ g of nitrocefin per ml. Fractions with high enzymatic activity were pooled, concentrated, and subjected to a QAE A50 anion-exchange column equilibrated with 20 mM TES [N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] buffer, pH 8.3. Eluents with enzyme activity were then pooled and concentrated. The purity of the enzyme was checked by using sodium dodecyl sulfate (SDS)-12% polyacrylamide gel electrophoresis (PAGE), as described previously (9).

The enzyme hydrolysis rates of purified CTX-M-5 were determined in 50 mM PO₄, pH 7.0, with a Beckman DU7400 spectrophotometer. For each substrate, the initial velocities were determined at six to eight substrate concentrations. The kinetic parameters k_{cat} and K_m were expressed as means of at least two independent kinetic evaluations and were calculated by the computer program ENZPACK (Biosoft; Elsevier). The standard deviation of each parameter was less than 20%. For the inhibition study, enzyme and inhibitor were preincubated in a volume of 50 μ l for 10 min at 25°C before the addition of 50 μ g of nitrocefin per ml (final volume, 1,000 μ l). Initial rates of hydrolysis were monitored spectrophotometrically at 495 nm, and 50% inhibitory concentrations (IC₅₀) were determined graphically.

Nucleic acid techniques. DNA isolation, restriction enzyme digestions, recombinant DNA manipulations, and transformations of plasmid DNA were performed as described by Sambrook et al. (27). Plasmids conferring ampicillin resistance were transferred from the clinical isolates to an ampicillin-susceptible, kanamycin-resistant *E. coli* host, strain DH5 α /pCLL2300, by filter mating (28). Transconjugants were selected on Luria-Bertani agar plates containing 100 μ g of ampicillin per ml and 30 μ g of kanamycin per ml. Plasmids conferring cefotaxime resistance were transformed into *E. coli* DH5 α by using CaCl₂ (27). SHV-1 β -lactamase was detected by PCR and subsequent *Nhe*I digestion, as described previously (23).

To facilitate nucleotide sequencing, a 7-kb *Bam*HI fragment encoding the β -lactamase with a pI of 8.8 from *S. typhimurium* 34 was cloned from an alkaline lysis plasmid DNA preparation into pCLL2300, a kanamycin resistance-conferring cloning vector (24), and the resulting plasmid was designated pCLL3417. Because the pI 8.8 β -lactamase present in these strains appeared to be phenotypically similar to the CTX-M-1 and CTX-M-2 β -lactamases, sequencing was initiated with 17-bp forward (5'-TCTGACGCTGGGTAAAG-3') and reverse (5'-CTTTACCCAGCGTCAGA-3') primers derived from a consensus region of the sequences of these two β -lactamase genes (5). Subsequently, both strands of the entire β -lactamase gene were sequenced with a nested set of custom-synthesized oligonucleotide primers which were specific for the CTX-M-5 β -lactamase gene. Initial DNA sequencing was performed on double-stranded plasmid DNA with a Sequenase kit (United States Biochemical, Cleveland, Ohio) with ³⁵S-ATP label (Amersham, Arlington Heights, Ill.) and completed by dideoxy sequencing with a *Taq* dye terminator kit (Applied Biosystems, Inc., Foster City, Calif.), according to the respective manufacturers' instructions.

Nucleotide sequence accession number. The nucleotide sequence data for *bla*_{CTX-M-5} was submitted to the GenBank nucleotide sequence database and assigned accession no. U95364.

RESULTS

Characterization of clinical isolates. The pIs of the β -lactamases present in crude extracts obtained from the clinical isolates are shown in Table 1. The clinical isolates expressed a β -lactamase with a pI of approximately 8.8. In addition, strain 34 produced a second enzyme with a pI of 7.6, which correlated with SHV-1. The presence of a non-ESBL SHV-type (most likely SHV-1) enzyme in the clinical isolates was confirmed in this strain by PCR with SHV-1 specific oligonucleotides and subsequent digestion with *Nhe*I (data not shown).

Plasmid profiles of strains 28 and 34 revealed multiple small plasmids ranging in size from 2 to 10 kb. In addition, strain 34 had one large plasmid (>50 kb). Mating experiments transferred the single large plasmid from *S. typhimurium* 34. This transconjugant (DH5 α /pCLL3422 + pCLL2300) expressed only the pI 7.6 β -lactamase. Transforming plasmid DNA from strain 34 resulted in transformant DH5 α /pCLL3425, which contained a single 10-kb plasmid and expressed only the pI 8.8 β -lactamase.

Susceptibility. MICs for the clinical isolates, transformant, transconjugant, and clone are listed in Table 2. With NCCLS criteria for intermediate and resistant used to define clinical resistance, the clinical isolates of *S. typhimurium* were resistant to the penicillins, amoxicillin-clavulanate, ampicillin-sulbactam, ticarcillin-clavulanate, cefotaxime, and aztreonam. The clinical isolates were susceptible to imipenem, cefoxitin, and ceftazidime, although the MICs of the latter were increased. *S. typhimurium* 28, which lacks the pI 7.6 β -lactamase, was susceptible to piperacillin-tazobactam. In addition, the clinical isolates of *S. typhimurium* were also resistant to chloramphenicol and trimethoprim-sulfamethoxazole but were susceptible to gentamicin, kanamycin, and ciprofloxacin (data not shown).

The pI 7.6 β -lactamase plasmid present in transconjugant strain DH5 α /pCLL3422 + pCLL2300 conferred resistance to the penicillins, amoxicillin-clavulanate, ampicillin-sulbactam, and ticarcillin-clavulanate. The pI 8.8 β -lactamase in strains DH5 α /pCLL3425 and DH5 α /pCLL3417 conferred resistance to cefotaxime and aztreonam, the penicillins, and the β -lactamase inhibitor combinations except for piperacillin-tazobactam. Although MICs of ceftazidime were increased, they were not increased to the level that would be considered resistant clinically (21).

β -Lactamase characterization. After two steps of column chromatography, the purified CTX-M-5 enzyme yielded a major single band (95% homogeneity) in both IEF and SDS-12% PAGE. From SDS-PAGE, the molecular weight of the CTX-M-5 β -lactamase was estimated to be 29,000 based upon Bio-Rad low-molecular-weight markers. The isoelectric point of

TABLE 2. MICs of β -lactam drugs for *S. typhimurium* clinical isolates and *E. coli* derivatives

Strain	MIC ($\mu\text{g/ml}$) ^a											
	AMP	AMC	SAM	TIC	TIM	PIP	PTZ	CAZ	CTX	ATM	IPM	FOX
<i>S. typhimurium</i> strains												
28	>128	16	64	>128	128	>128	2	8	128	32	0.25	2
34	>128	32	64	>128	>128	>128	64	8	128	32	0.25	2
<i>E. coli</i> strains												
DH5 α	8	8	8	4	1	1	0.25	0.25	≤ 0.12	≤ 0.12	0.25	8
DH5 α /pCLL2300	8	8	8	4	1	1	0.25	0.25	≤ 0.12	≤ 0.12	0.25	8
DH5 α /pCLL3422 + pCLL2300	>128	64	32	>128	128	128	8	1	0.5	0.5	0.5	8
DH5 α /pCLL3425	>128	32	32	>128	128	>128	1	16	>128	128	0.5	16
DH5 α /pCLL3417	>128	32	64	>128	128	>128	0.5	16	>128	128	0.5	8

^a MICs were determined in broth microdilution tests (21). AMP, ampicillin; AMC, amoxicillin-clavulanate; SAM, ampicillin-sulbactam; TIC, ticarcillin; TIM, ticarcillin-clavulanate; PIP, piperacillin; PTZ, piperacillin-tazobactam; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; IPM, imipenem; FOX, ceftioxin.

homogeneously purified CTX-M-5 was 8.8, consistent with the enzyme produced from the wild-type strain.

The substrate and inhibition profiles are shown in Table 3. CTX-M-5 β -lactamase showed broad-spectrum hydrolytic activity against penicillin, cephalosporins, and aztreonam. Cefotaxime was a good substrate for CTX-M-5, with comparable hydrolytic activity (k_{cat} , 210 s^{-1}) and affinity (K_m , $77 \mu\text{M}$) to those of benzylpenicillin. Cephaloridine was also a good substrate, with higher hydrolytic activity but lower affinity. Ceftazidime was readily hydrolyzed by the CTX-M-5 enzyme but had a lower rate (<5%) and lower affinity (higher K_m) than those of cefotaxime or benzylpenicillin. The physiological efficiency (k_{cat}/K_m) of CTX-M-5 for ceftazidime was only 6% of that of cefotaxime. Aztreonam was also hydrolyzed by CTX-M-5 with a lower rate and lower affinity than cefotaxime. Cefoxitin and imipenem were poor substrates for CTX-M-5, with relative hydrolysis rates of <0.1% of that of benzylpenicillin. Clavulanic acid, tazobactam, and sulbactam were all effective β -lactamase inhibitors for the CTX-M-5 β -lactamase, with IC_{50} s of 19, 100, and 3.4 nM respectively. CTX-M-5 is a cefotaxime-hydrolyzing β -lactamase which is inhibited by clavulanate and is therefore classified in functional group 2be.

Nucleotide sequencing. The nucleotide sequence of *bla*_{CTX-M-5} is given in Fig. 1, and the derived amino acid sequence is given in Fig. 2. The *bla*_{CTX-M-5} β -lactamase gene is 879 bp long, and the encoded protein consists of 293 amino acids. It is 63.2% identical to the CTX-M-1 β -lactamase gene and 97.5% iden-

tical to the CTX-M-2 β -lactamase gene (5). There is similar homology on the protein level. The nucleotide sequence of *bla*_{CTX-M-5} differs from *bla*_{CTX-M-2} by 11 nucleotides, 4 of which give rise to amino acid changes: threonine for alanine 27, glycine for valine 230, alanine for glutamate 254, and valine for isoleucine 278.

DISCUSSION

A serious outbreak of *S. typhimurium* in Latvia was further complicated by the fact that the outbreak strain was resistant to multiple antibiotics, including ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and cefotaxime, which are commonly used to treat serious infections caused by *Salmonella* spp. These strains were uniformly susceptible to ciprofloxacin; however, the extremely young ages of most of the patients prevented its use in treatment.

The isolates described in this report were resistant to cefto-

TABLE 3. Substrate and inhibitor profiles of purified CTX-M-5 β -lactamase

Substrate or inhibitor	k_{cat} (s^{-1})	Relative k_{cat}	K_m (μM)	k_{cat}/K_m ($\text{mM}^{-1}\text{s}^{-1}$)	IC_{50} (nM)
Substrates					
Benzylpenicillin	230	100	50	4,600	
Cephaloridine	1,500	680	350	4,400	
Cefotaxime	210	95	77	2,800	
Ceftazidime	7.4	3.3	440	17	
Aztreonam	21	9.3	730	29	
Cefoxitin	0.41	0.18	NC ^a	NC	
Imipenem	0.0027	0.0012	NC	NC	
Inhibitors					
Clavulanic Acid					19
Sulbactam					100
Tazobactam					3.3

^a NC, not calculated (rates were too low to obtain reliable values).

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TTTTTATGAT GACTCAGAGC ATTCGCCGCT CAATGTTAAC GGTGATGGCG 50
ACGCTACCCC TGCTATTTAG CAGCGCAACG CTGCACGGCG AGACGAACAG 100
CGTGACGACG CAGCTGGAAG CCTGGAGAA AAGTTCGGGA GGTCCGCTTG 150
GCGTTGCGCT GATTAACACC GCCGATAATT CGCAGATICT CTACCGTGCC 200
GATGAACGTT TTGCGATGTG CAGTACCAGT AAGGTGATGG CGGCCGCGCG 250
GGTGCTTAAA CAGAGCGAGA GCGATAAGCA CCTGCTAAAT CAGCGCGTTG 300
AAATCAAGAA GAGCGACCTG GTTAACCTACA ATCCCATIGC GGAGAAACAC 350
GTTAACGGCA CGATGACGCT GGCTGAGCTT GGCGCAGCGG CGCTGCAGTA 400
TAGCGACAAT ACTGCCATGA ATAAGCTGAT TGCCCATCTG GGTGGGCCCG 450
ATAAAGTGAC GCGCTTTGCT CGCTCGTTGG GTGATGAGAC CTTCCGTTCTG 500
GACAGAACCG AGCCACGCT CAATACCGCC ATCCAGCGCG ACCCGCGTGA 550
TACCACCACG CCGCTCGCGA TGCGCGACAG CCTGAAAAAT CTGACGCTGG 600
GTAAAGCGCT GCGGGAAACT CAGCGGGCAC AGTTGGTGAC GTGGCTTAAG 650
GGCAATACTA CCGGTAGCGC GAGCATTCCG GCGGGTCTGC CGAAATCATG 700
GGGAGTGGGC GATAAAACCG GCAGCGGAGA TTATGGCACC ACCAACGATA 750
TCGCGGTTAT CTGGCCGCGA AACCCACGAC CGCTGGTTCT GGTGACCTAC 800
TTTACCACAC CGGAGCAGAA GCGGGAAGC CGTCGGGATG TTCTGGCTGC 850
GGCGCGGAAA ATCGTAACCC ACGGTTTCTG ATGCAATAAA TGGAGCGAGG 900
TATCGCTCCA TTTACGTTAA AATGTGCTC CTGAACCTCA GTCTGTCTTT 950
GCAACGAACC CTCATCTCTT TCTATCATCC CGCTCTTTTT TCTGAACCAA 1000
ATAGCTTAGC TTCAGGAAG TAATAGATA CAATTTCTTA TGTAACATAT 1050

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FIG. 1. Nucleotide sequence of the *bla*_{CTX-M-5} β -lactamase gene. Underlined nucleotides are start and stop codons.

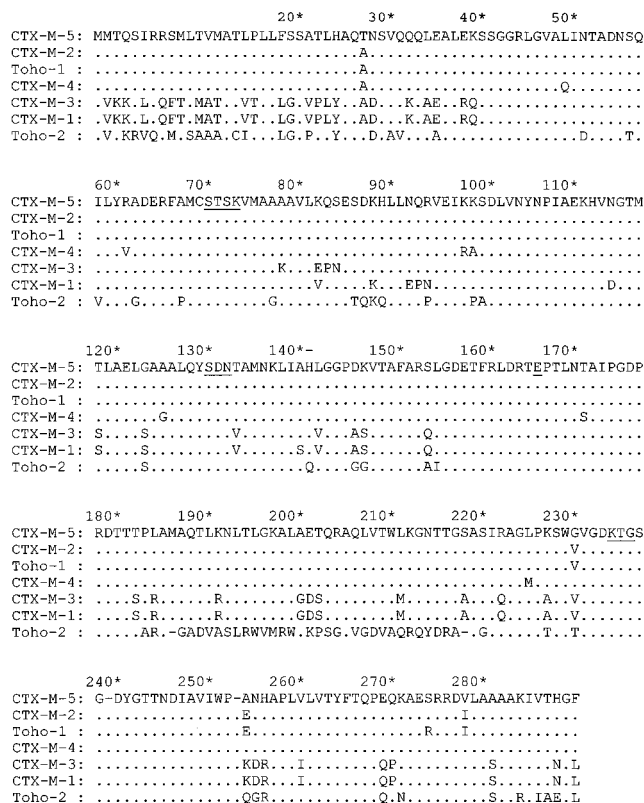


FIG. 2. Derived amino acid sequence of the CTX-M-5 β-lactamase compared to the cefotaxime-hydrolyzing β-lactamases CTX-M-1, CTX-M-2 (5), CTX-M-3 (13), CTX-M-4 (12), Toho-1 (15), and Toho-2 (18). Dots indicate identical amino acids; underlining indicates the 70SXXK⁷³, 130SDN¹³², E-166, and 234KTG²³⁶ conserved sequences that are typical of class A serine β-lactamases. Amino acid numbering is according to the scheme outlined by Ambler et al. (1).

taxime due to the presence of a novel extended-spectrum β-lactamase, CTX-M-5. It is interesting that in this small family of cefotaxime-hydrolyzing β-lactamase, CTX-M-2, which is the enzyme most closely related to CTX-M-5, and CTX-M-4 also originated in *S. typhimurium* (3, 12). The strains of *S. typhimurium* described by Bauernfeind et al. from which CTX-M-2 was detected were isolated from an outbreak of severe infections in Buenos Aires, Argentina, in 1990 (3). Therefore, it is unlikely that the Argentinean strains are related to the Latvian strains described in this study. Further observations of *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* strains expressing CTX-M-2 were made in Germany, Israel, and Paraguay (5). The other members of the CTX-M family of β-lactamases were found in *E. coli* and *Citrobacter freundii* strains isolated in Germany, Italy, Japan, Poland, and Russia (2, 4, 12, 13, 15). The wide geographical distribution of occurrences of these β-lactamases implies that they represent independent genetic events; however, it is interesting that CTX-M-3, CTX-M-4, and CTX-M-5 were all found in isolates from Eastern European countries (12, 13). To date, all of the strains expressing CTX-M-type enzymes have carried the β-lactamase on a plasmid. The origin of the enzymes remains unknown, although they are most closely related to the chromosomally mediated β-lactamase from *Klebsiella oxytoca* (5, 25).

The CTX-M family of ESBLs is interesting in that it has uniquely potent hydrolytic activity against cefotaxime. Like the cefotaxime hydrolyzing β-lactamases CTX-M-1, CTX-M-2,

and Toho-1 (3, 4, 15), CTX-M-5 has more potent activity against cefotaxime than against ceftazidime. This finding confirms the analysis of the relationship between compound structure and enzymatic stability by Bauerfeind et al., who concluded that the carboxy-isopropoximino group of ceftazidime protects the compound from attack by the CTX-M-type enzymes, whereas the methoximino group of cefotaxime provides the compound better affinity to the enzyme (4). The enzyme kinetic data for CTX-M-2 (3), which is most closely related to CTX-M-5, indicated a hydrolytic pattern similar to that of CTX-M-5, in that cephaloridine is a better substrate than benzylpenicillin or cefotaxime. Toho-1 (15), which differs from CTX-M-2 by only two amino acid residues, hydrolyzed cephaloridine and cefotaxime much faster (10- to 16-fold) than benzylpenicillin. These differences in substrate profiles might also represent experimental differences between various investigators' laboratories.

The combination of CTX-M-5 and SHV-1 in three of the clinical isolates studied conferred resistance to piperacillin-tazobactam. However, clinical isolates or laboratory constructs producing only one of these enzymes were susceptible to this β-lactam-β-lactamase inhibitor combination. It appears that the combination of SHV-1 plus an ESBL can confer resistance to piperacillin-tazobactam, whereas strains expressing the ESBL alone are often susceptible (7, 8, 16, 30). The resistance to piperacillin-tazobactam in these strains expressing multiple β-lactamases is most likely attributable to the SHV-1 enzyme, which is not inhibited as well by β-lactamase inhibitors as most other class A enzymes (11).

The prevalence of this cefotaxime-hydrolyzing β-lactamase among *S. typhimurium* strains in Latvia may be related to the increased usage of expanded-spectrum cephalosporins in recent years; however, it is difficult to assess whether or not this resistance developed as a direct result of cephalosporin therapy. With the recognition of the high prevalence of resistance, efforts have been made to curtail antibiotic use in patients with salmonellosis, and efforts to control the spread of *S. typhimurium* are under way. The detection of a new member of the CTX-M family in Latvia raises important questions about whether such enzymes arise de novo in multiple geographic locations or, alternatively, are transmitted across national borders. The importance of these questions emphasizes the need for global surveillance of antibiotic resistance so that appropriate control strategies can be designed and implemented.

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